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Acidobacteria Phylum Sequences in Uranium-Contaminated Subsurface Sediments Greatly Expand the Known Diversity within the Phylum▽†

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The abundance and composition of bacteria of the phylum *Acidobacteria* were surveyed in subsurface sediments from uranium-contaminated sites using amplification of 16S rRNA genes followed by clone/sequence analysis. Analysis of sequences from this study and public databases produced a revised and greatly expanded phylogeny of the *Acidobacteria* phylum consisting of 26 subgroups.

Bacteria in the *Acidobacteria* phylum have been detected by 16S rRNA gene-based surveys in a wide variety of environments, although little is known about their physiology or ecology. Over 3,000 sequences represent this phylum in public databases, and almost all of them were obtained from uncultivated organisms from very diverse environments (4). In soils and sediments, the *Acidobacteria* appear to be abundant, comprising 10 to 50% of 16S rRNA gene sequences in clone libraries from materials that vary greatly in physical, geochemical, and biological characteristics (2, 7, 10). The ubiquity, diversity, and abundance of *Acidobacteria* phylum members in soils and sediments, and their ability to withstand metal-contaminated, acidic, and other extreme environments, prompted us to determine their relative abundance and composition in subsurface sediments contaminated with uranium (U) and other toxic materials. We present here the wide diversity of *Acidobacteria* phylum sequences present in U-contaminated subsurface sediments and a comprehensive and greatly

TABLE 1. Phylum level composition of 16S rRNA gene libraries from uranium-contaminated sites in the United States[a]

Parameter	Site										
	Tennessee							Colorado			
	Background area		Area 1		Area 2			Up-gradient		Down-gradient	
	FB300	FB605	TPB10	FW015	TPB15[g]	TPB16	DP13	G07	G16	P02	P03
% of clones of bacterial phylum											
Proteobacteria	**15.0**	**38.7**	**14.0**	3.5	6.9	**11.1**	**11.9**	**24.6**	**29.4**	**32.1**	**39.7**
Chloroflexi	2.7	1.3	**20.2**	**20.2**	5.1	4.7	**14.3**	**18.0**	**17.6**	**12.5**	**14.7**
Actinobacteria	**11.0**	6.7	**10.1**	**16.7**	3.5	7.9	2.4	6.6	**17.6**	**10.7**	**10.3**
Acidobacteria	**17.8**	**20.0**	9.0	**15.5**	1.7	3.2	7.1	0	**11.8**	7.1	2.9
Firmicutes	4.1	2.6	5.1	1.2	0	**52.4**	0	3.3	2.0	**14.3**	8.8
Chlorobium	0	2.6	3.8	2.6	**69.0**	0	4.8	8.2	2.0	1.8	7.4
Verrucomicrobia	5.5	4.0	1.2	0	3.5	0	**14.3**	**13.1**	5.9	0	1.5
Gemmatimonadetes	2.7	0	6.3	7.1	1.7	0	0	0	0	3.6	1.5
Incertae sedis[b]	5.5	4.0	3.8	0	6.9	1.6	0	6.6	3.9	3.6	1.5
Other	**12.3**	9.3	6.3	6.3	0	3.2	0	4.9	5.9	8.9	2.9
Unclassified[c]	**23.3**	**14.6**	**20.2**	**26.2**	1.7	**15.9**	**45.2**	**14.7**	3.9	5.4	8.8
Total no. of clones	73	75	79	84	58	63	42	61	51	56	68
Total no. of sequence types[d]	73	75	78	73	29	61	42	61	51	56	68
Phylum richness[e]	12	11	10	9	8	7	6	10	7	12	11
Library coverage[f]	1	1	1	1	2	1	1	1	1	1	1

[a] Cells containing >10% of sequences for that library are in boldface type.
[b] Genera insertae sedis includes members of the candidate phyla TM7, WS3, OP10, and OP11.
[c] Unclassified sequences could not be placed into any known phylum with high confidence.
[d] Total number of sequence types determined using the FastGroup program as described in the supplemental material.
[e] Phylum richness is the number of divisions identified in the clone library not including sequences in the "unclassified" category.
[f] Library coverage is the number of clones divided by the number of sequence types.
[g] This sample is located downstream of a reactive barrier for U removal from groundwater.

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expanded phylogeny of this phylum that includes new subgroups dominated by sequences from U-contaminated materials.

U-contaminated subsurface sediments were obtained from U.S. Department of Energy sites in Tennessee and Colorado (see Table S1 in the supplemental material) (3). The Tennessee sediments are acidic and contaminated with U, technecium, other metals, nitrate, and other organic contaminants (www.esd.ornl.gov/nabirfrc) (5, 9, 15, 17). Sediments from the Colorado site are contaminated only with U at lower concentrations and neutral pH (http://web.em.doe.gov/bemr96/ners.html) (1, 16, 19).

Nucleic acids were extracted, purified, and quantified from triplicate 30-g sediment samples (8). DNA yields were low, 2 to 11 ng/g sediment from the Tennessee samples and 7 to 42 ng/g sediment from the Colorado sediments, and reflected the low biomass and cell count data obtained from some of these samples (5, 6).

16S rRNA gene sequence surveys were conducted using primers 27F (13) and 787R (3, 12) to determine the relative contribution of *Acidobacteria* sequences to the total bacterial community (Table 1). Sequences were assigned to bacterial phyla based on comparisons to database sequences (http://simo.marsci.uga.edu/public_db/rdp_query.htm) and phylogenetic analyses. Despite the low biomass, bacterial sequence diversity was very high in the subsurface sediments (coverage values in Table 1). At least 18 bacterial phyla were detected, with the *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, and *Acidobacteria* being the most well-represented phyla. In Colorado sediments, 5.1% of the sequences were from the *Acidobacteria*. In Tennessee, a significantly higher percentage (t test, $P = 0.003$) of *Acidobacteria* sequences was found in uncontaminated background sediments ($\bar{x}$, 18.9%; standard deviation [SD], 1.5; $n = 2$) than in the contaminated sediments ($\bar{x}$, 7.3%; SD, 5.4; $n = 5$).

Acidobacteria rRNA gene libraries were prepared using the phylum-specific primers 31F and 787R (2, 3). Phylogenetic analyses (maximum likelihood, distance matrix, and maximum parsimony methods are described in the supplemental material) were conducted on sequences generated from this study ($n = 700$) aligned with sequences representative of phylum diversity obtained from public databases ($n = 570$) (Fig. 1).

The *Acidobacteria* phylum was originally described as having four to five major subgroups based on 16S rRNA gene sequences available at the time (11, 14). This was expanded to 8 subgroups the following year (7) and to 11 subgroups in 2005 (20), as sequences from an increasing number of 16S rRNA gene surveys became available. Analysis of sequences obtained in this study, together with those available in the database, considerably expands and updates the known diversity within the *Acidobacteria* phylum and provides a framework for further classification of species within this phylum. Trees obtained in the present analyses define at least 26 sequence subgroups, most of which are well supported by bootstrap analyses (Fig. 1). An effort was made to extensively sample sequence diversity in the databases, but very few sequences were found to fall outside the 26 observed subgroups. Although partial 16S rRNA gene sequences were used, the high bootstrap support obtained for most groupings, using several phylogenetic analysis methods, indicates that sufficient data were available to reliably resolve the relationships within the phylum.

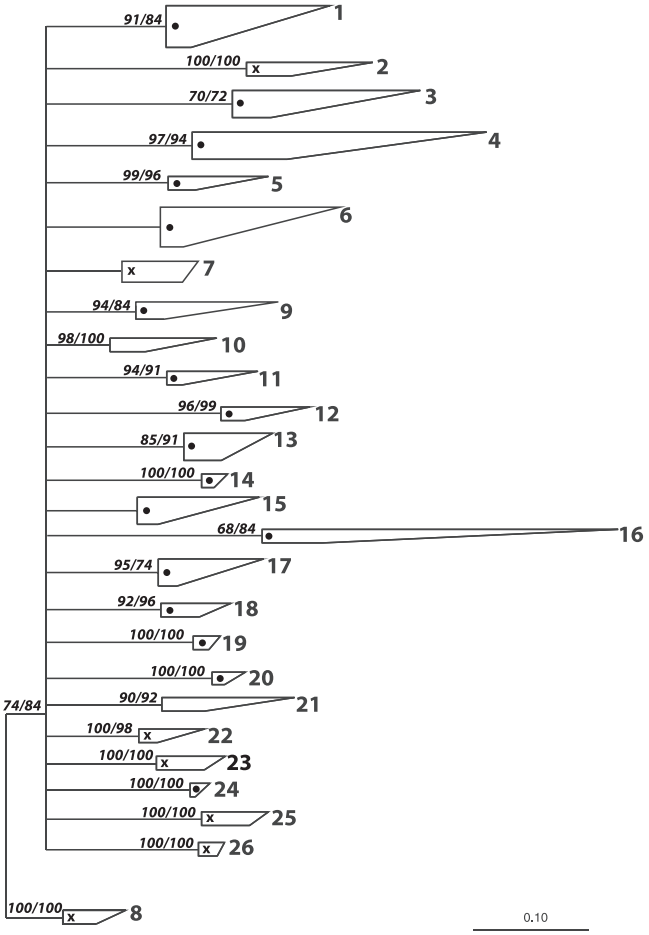

FIG. 1. Schematic tree of *Acidobacteria* phylum 16S rRNA gene sequence diversity based on maximum likelihood analysis of 405 sequences representative of types found in the present study (214 sequences) and in the database (191 sequences). Bootstrap support for the monophyly of groups determined by analysis of phylogenetic representatives of the sequences (before slash) versus that for all 700 of the sequences (after slash) is shown for results >70% by one or both analyses. Subgroups 1 to 8 are those described previously by Hugenholtz et al. (7), while groups 9 to 11 are those described previously by Zimmerman et al. (20). Numbering of groups above 11 was arbitrary. The shape of group "wedges" is indicative of sequence diversity observed within the group. Less than 70% bootstrap support was obtained for the order of branching of any groups above group 8 in the tree, as indicated by the single vertical line connecting the groups. The tree was rooted using the sequence of *Escherichia coli* as the outgroup. Subgroup wedges containing dots contain sequences from the U-contaminated sediments in this study. Subgroup wedges containing an "x" are those subgroups not detected using primer 31F.

The *Acidobacteria* sequences from the subsurface sediments were very diverse, clustering into 17 of the 26 subgroups (Table 2 and Fig. 1). Sequences from this study fell into all previously identified subgroups within the phylum (detectable with this primer set) (subgroups 1, 3 to 6, 9, and 11) (7, 20) and also clustered into 10 new, previously unpublished subgroups. Three of the new subgroups are composed entirely of sequences obtained in this study (subgroups 12, 20, and 24). Each of the 17 subgroups contained sequences from at least two

TABLE 2. Diversity and composition of abundant *Acidobacteria* phylum members in uranium-contaminated sites in the United States[a]

Parameter	Site									
	Tennessee						Colorado			
	Background area		Area 1	Area 2			Up-gradient		Down-gradient	
	FB300	FB605	TPB10	TPB15	TPB16	DP13	G07	G16	P02	P03
% of clones (actual no. of clones) for subgroup[b]:										
1	**19.6 (19)**	**31.0 (22)**	2.7 (2)	9.0 (6)	**18.8 (15)**	**13.0 (9)**	0 (0)	1.7 (1)	1.4 (1)	2.9 (2)
3	**17.6 (17)**	**19.7 (14)**	8.2 (6)	**16.4 (11)**	1.2 (1)	5.8 (4)	0 (0)	**18.3 (11)**	1.4 (1)	1.4 (1)
4	7.2 (7)	**17.0 (12)**	0 (0)	3.0 (2)	0 (0)	5.8 (4)	**18.6 (8)**	**10.0 (6)**	2.9 (2)	**11.4 (8)**
5	3.1 (3)	5.6 (4)	0 (0)	6.0 (4)	0 (0)	**10.1 (7)**	0 (0)	0 (0)	0 (0)	0 (0)
6	**40.2 (39)**	**12.7 (9)**	**13.7 (10)**	**22.4 (15)**	0 (0)	7.2 (5)	**16.3 (7)**	**26.7 (16)**	**30.0 (21)**	**41.4 (29)**
12	0 (0)	0 (0)	**34.3 (25)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.4 (1)	0 (0)
13	3.1 (3)	**11.3 (8)**	4.1 (3)	**16.4 (11)**	8.8 (7)	**21.7 (15)**	2.9 (2)	5.0 (3)	1.4 (1)	2.9 (2)
14	0 (0)	0 (0)	0 (0)	3.0 (2)	**66.3 (53)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15	1 (1)	1.4 (1)	2.7 (2)	**11.9 (8)**	0 (0)	0 (0)	**25.6 (11)**	**11.6 (7)**	**27.1 (19)**	**10.0 (7)**
17	4.1 (4)	1.4 (1)	**24.7 (18)**	0 (0)	0 (0)	4.4 (3)	**16.3 (7)**	**15.0 (9)**	**21.4 (15)**	**14.3 (10)**
24	0 (0)	0 (0)	0 (0)	3.0 (2)	0 (0)	**26.1 (18)**	0 (0)	0 (0)	0 (0)	0 (0)
Total no. of clones	97	71	73	67	80	69	43	60	70	70
Total no. of sequence types[c]	82	60	36	44	14	31	35	54	65	64
Total no. of subgroups	10	8	10	10	7	10	9	11	13	12
Coverage[d]	1.2	1.2	2	1.5	5.7	2.2	1.2	1.1	1.1	1.1

[a] Cells containing >10% of the sequences for a library are in boldface type.
[b] Only subgroups for which at least one sediment library contained >10% members in that subgroup. See reference 3 for a full tally.
[c] Total number of sequence types determined using the Fastgroup program as described in the supplemental material.
[d] Library coverage is the number of clones divided by the number of sequence types.

distinct samples. Within some subgroups, nearly identical sequences were identified from both the Tennessee and Colorado sites (e.g., in subgroups 13 and 15 to 18). This is interesting because the parent sediment materials, geochemistries, and contaminant compositions and concentrations differ greatly between these two sites.

The number of subgroups represented in the *Acidobacteria* sequence libraries was similar between U-contaminated sediments ($n = 7$ to 10) and uncontaminated background samples ($n = 8$ to 10) in Tennessee. The number of subgroups in the Colorado samples was also similar ($n = 9$ to 13). However, the number of sequence types present in the U-contaminated samples in Tennessee was reduced relative to those in the uncontaminated background samples (Table 2) (fewer total sequence types and coverage values two- to fivefold higher), suggesting that the conditions in the contaminated sites may be optimal for fewer species that can tolerate the low-pH, high-nitrate, and high-radionuclide conditions.

Acidobacteria phylum members have been found through 16S rRNA gene surveys in other U-contaminated materials. In Germany and Colorado, the *Acidobacteria* comprised 26% and 9% of the bacterial 16S rRNA gene sequences, respectively (18). In our analysis, sequences from these sites were found to be members of subgroups 1 to 3, 5, 6, 10, and 13. The finding of similar 16S rRNA gene sequences in very different materials contaminated with U raises the question of whether there are certain *Acidobacteria* species that may be widely distributed in radionuclide-contaminated environments.

Acidobacteria subgroups 1, 3, 4, and 6 are the most abundant subgroups in soil surveys worldwide (2, 10). For example, in 16S rRNA gene surveys of two soil samples in New Mexico and Utah, subgroups 4 and 6 comprised 42.8% and 45.6% of sequences in *Acidobacteria* 16S rRNA gene libraries ($n = 9$ libraries, with a total of 673 sequences) (unpublished results). Similarly, the uncontaminated sediments in Tennessee were dominated by these four subgroups ($\bar{x}$, 87%; SD, 1.4; $n = 2$). In contrast, the contaminated sediments contained a significantly lower percentage of these subgroups ($\bar{x}$, 44%; SD, 14; $n = 8$; t test, $P = 0.018$) and a higher representation of the new subgroup sequences. The metabolic capabilities and ecological roles that these bacteria play in U-contaminated sites, or in any environment, are still unknown, and our results present an interesting correlation for further study.

Nucleotide sequence accession numbers. Sequences representative of each new subgroup type obtained in this study have been deposited in GenBank under accession numbers EF457296 to EF457509.

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